# PHYSIOLOGY OF INSECT DIAPAUSE. XIV. AN ENDOCRINE MECHANISM FOR THE PHOTOPERIODIC CONTROL OF PUPAL DIAPAUSE IN THE OAK SILKWORM, ANTHERAEA PERNYI

CARROLL M. WILLIAMS[1] AND PERRY L. ADKISSON[2]

*The Biological Laboratories, Harvard University, Cambridge 38, Massachusetts*

During the final ten days of larval life, the Pernyi silkworm envelops itself in a stout-walled cocoon within which it pupates. Development may stop right there as the pupa begins a prolonged period of pupal diapause which persists until the following spring. Alternatively, the newly formed pupa may develop into an adult moth without any delay. The moth is then committed to the reproduction of a further generation of pupae which can begin to diapause before the first killing frost. If winter arrives before the larvae can pupate, the insect will experience what is little short of "ecological suicide."

Like so many plants and animals, the Pernyi silkworm minimizes these ecological dangers by monitoring seasonal signals of utmost precision, namely, the lengths of the night and day. For *A. pernyi* the phenomenon is well documented in the detailed studies of the Japanese investigator, Tanaka (1950a, 1950b, 1950c; 1951a, 1951b; see Lees, 1955, for English summary). Thus, when Pernyi larvae are reared under day-lengths longer than 14 hours, they develop without any pupal diapause; at temperate latitudes, photoperiods of this sort are peculiar to late spring and early summer when the season is propitious for a second brood. By contrast, larvae reared under day-lengths shorter than 14 hours (as in late summer and autumn) transform into diapausing pupae.

Little is known about the physiological basis of the photoperiod response. In principle, the minimal mechanism must include, not only a photoreceptor, but also a clockwork-computer which counts the hours of darkness and daylight. Until recently, all that was known was Tanaka's finding that the larval ocelli are not involved in the reception of photoperiod.

But the induction of diapause is only half the story. Of equal significance is the termination of diapause—its timing and synchronization with the seasons.

In a related species, the Cecropia silkworm, the termination of pupal diapause is known to be controlled primarily by environmental temperature. By an apparently direct action on the brain itself, environmental temperature conditions the secretion of a hormone prerequisite for the termination of diapause and the initiation of adult development (Williams, 1956).

This same temperature-sensitive system has generally been presumed for other diapausing pupae including *A. pernyi*. Indeed, as pointed out in de Wilde's

[1] This study was supported, in part, by a grant from the National Science Foundation.

[2] Special Postdoctoral Fellow of the U. S. Public Health Service during the term of the study. Permanent address: Department of Entomology, Texas A & M University, College Station, Texas.

(1962) recent review, insect pupae (unlike all other stages in the life history) are thought to be insensitive to photoperiod.

Some reservations on this point are provoked by a more detailed examination of *A. pernyi*. Thus, when the cocoon is cut open, the pupa is always facing upward in the chamber. Moreover, on inspecting the pupa, itself, one cannot fail to be impressed by the unpigmented, transparent cuticle overlying the brain (Fig. 1). Even if the rest of the pupa is jet-black, the facial cuticle is always pigment-free. By moistening it with alcohol, one can look inside and see the underlying brain. A similar "facial window" is found in all pupae of the genus *Antheraea*, including the American species, *A. polyphemus*.

FIGURE 1. Two pupae of *A. pernyi* are here photographed alongside a Cecropia pupa. Pernyi routinely shows a zone of transparent cuticle overlying the pupal brain; the Cecropia pupa does not.

Is it possible that the window has something to do with the transmission of light? This prospect seems to have been reported only by the Russian investigator, Shakhbazov (1961).

Impressed by the facial window, one of us tested, some 16 years ago, the influence of illumination on diapausing pupae of *A. polyphemus*. Groups of pupae were removed from cocoons, subdivided into two lots, and placed at 25° C. in continuous light and darkness, respectively. Illumination had no detectable effects on the rate of termination of diapause.

Subsequently we have learned that continuous light or darkness are both

aperiodic signals which, in this sense, are inappropriate tests for photoperiodism. The experiment has now been repeated in a proper manner by making use of specific photoperiods. Certain of the results have been summarized previously (Williams, 1963; Williams and Adkisson, 1964).

## Materials and Methods

### 1. *Experimental animals*

All experiments were performed on pupae of *A. pernyi*. The cocoons were the diapausing first-brood harvested in late July; on August 27 they were shipped from Japan in a series of opaque cardboard boxes. They arrived at Harvard University on September 30.

One hundred pupae were removed from cocoons and inspected under the dissecting microscope to confirm the persistence of diapause. The rest of the cocoons were then subdivided into two lots. One group was spread on tables in a $25 \pm 0.5°$ C. room programmed for a daily illumination of 8 hours; the other group was stored in opaque boxes at 2–3° C.

A few experiments were performed on a subsequent shipment consisting of diapausing cocoons of the second generation harvested in October. This material was stored at 2–3° C.

### 2. *Photoperiod studies*

Chilled and unchilled cocoons were exposed for 16 weeks to precise regimens of photoperiod. Some pupae were removed from their cocoons at the outset of the experiment; the vast majority were not.

After the insects had been placed at the selected photoperiods, they were inspected at least twice weekly, the number of emerged moths being recorded. Pupae not enclosed in cocoons were examined under the dissecting microscope to ascertain whether adult development had begun. Needless to say, all observations (with the exception of the individuals maintained under continuous darkness) were performed during the photophase of the daily cycle. Chambers maintained in continuous darkness were opened in a dark room and inspected under red light.

### 3. *Adult development*

The initiation of adult development is signaled by the detachment and retraction of the epidermis from the pupal cuticle. The "zero day of development" was recognized by the initiation of retraction of the epidermis on the ventral side of the tip of the abdomen where the cuticle overlying the genital disc is usually palely pigmented. The overlying cuticle was moistened with 70% ethanol and viewed under a dissecting microscope. In order to eliminate surface reflections, observations of this type are facilitated if a Polaroid filter is placed in front of the microscope lamp and a second "crossed" filter is positioned under the objective lens (Harvey and Williams, 1958).

In pupae of pale pigmentation, the initiation of epidermal retraction was also visible in the pupal wings at the onset of adult development. But in the jet-black pupae which are sometimes encountered, observation of wing retraction was pos-

sible only when a zone of the superficial pigment layer was scraped away with a scalpel. A time-table for the adult development of Pernyi will be described in the section on Results.

4. *Experimental chambers*

Two types of chambers were employed. The first consisted of six B.O.D. incubators modified so that each of the three shelves was illuminated from directly overhead by a 15-watt fluorescent lamp (Sylvania cool white, F15T8). The average intensity of illumination was approximately 175 foot-candles (1883 lux).

The second type of chamber was an adaptation of that described by Dutky *et al.* (1962). Five-gallon tin-cans with tight-fitting lids were employed. A 4-watt fluorescent lamp (General Electric cool white F4T5) was installed in the lid of each can to yield an average internal illumination of 110 foot-candles (1184 lux). An electrically driven exhaust fan was attached to the lid and an air-intake placed near the bottom; the intake and exit ports were fitted with coiled lengths of radiator hose to prevent any leakage of light. The assembled chambers were placed in a constant-temperature room at $25 \pm 0.5°$ C. and a relative humidity of 60%. It may be noted that these simple chambers were in every way as satisfactory as the expensive B.O.D. incubators.

The daily cycle of illumination was programmed for each chamber by a Model No. 8001 "Tork" time-clock. In the case of the B.O.D. incubators, the timer's double-throw switch was used to turn on a 50-watt heater (positioned in the rear of the lower shelf) whenever the fluorescent lamps were turned off. This arrangement minimized the temperature fluctuations occasioned by the operation of the lamps.

In all cases (except, of course, the chambers maintained in continuous light or dark) the chambers were programmed for 24-hour daily cycles of light and darkness. For convenience, we shall identify each photoperiod in terms of the duration of the daily light-cycle or "photophase." By so doing, we automatically define the duration of dark-cycle or "scotophase." This terminology seems most straight-forward despite the fact that the length of the night is probably more crucial than the length of the day (Adkisson, 1964).

5. *Photoperiod gradients*

In order to expose opposite ends of individual pupae to different photoperiods, two simple mechanisms were utilized. The first of these consisted of a block of wood in which a series of circular holes, 15 mm. and 17 mm. in diameter, was drilled. A second board was screwed to the bottom of the first to seal the lower ends of the holes. All surfaces were painted with a non-reflecting black paint.

Pupae were selected of appropriate diameters to slip snugly into the holes by friction-fit. Half faced upwards in the holes; half downwards. The entire assembly was placed in a 25° C. incubator programmed for an 8-hour photophase. In this manner one end of each individual was exposed to the 8-hour photophase while the opposite end was maintained in continuous darkness.

In later experiments the set-up was modified to permit one to establish specific photophases on both upper and lower surfaces. For this purpose six rows of

holes were drilled through a 15 × 40 × 4 cm. board. The top side was framed to receive a tight-fitting removable lid and all surfaces were painted black.

As described above, the holes were plugged with pupae and the entire assembly placed in a 25° C. incubator programmed for a 16-hour daily photophase. With the lid removed, both upper and lower surfaces were illuminated by the incubator's fluorescent lamps above and below the assembly. After 8 hours of the 16-hour photophase, the lid was sealed in place. Sixteen hours later, at the beginning of the next photophase, the lid was removed. This cycle was repeated daily. The net effect was to expose the upward-facing ends to an 8-hour photophase while the downward-facing ends of the same individuals received a 16-hour photophase.

## Experimental Results

### 1. *Time-table for adult development at 25° C.*

As described under Methods, the "zero day of adult development" was recognized in terms of the initiation of retraction of the epidermis. We would emphasize that regardless of how many days, weeks, or months are required for the initiation of adult development, the latter, once begun, then proceeds at a rate dictated almost entirely by environmental temperatures. (See below under 7C.)

In Table I we present an abridged version of a time-table for adult development at 25° C. Characters singled out are in most cases visible in the intact animal under the dissecting microscope. When maintained at 25° C., the moths emerged 19 ± 2 days after the visible initiation of development. The latter, as signaled

Table I

*Time-table for the adult development of Antheraea pernyi at 25°C, with special reference to externally visible characters*

| Day | Characters |
|---|---|
| 0 | The epidermis begins to retract from the overlying pupal cuticle in the wings and at the underside of the tip of abdomen; no visible retraction of leg epidermis. |
| 1 | Full retraction of the epidermis of wings and tip of abdomen; facial retraction present only along posterior margin and in posterior angles; trace of leg retraction. |
| 2 | Full retraction of leg epidermis. |
| 7 | Compound eyes fully faceted and show initiation of pale pink pigmentation; genitalia fully formed but show no silky pubescence. |
| 9 | Brown pigmentation of eyes. Genitalia covered with silky pubescence but cuticle remains unpigmented. |
| 10 | Dark brown pigmentation of eyes; genitalia remain covered with silky hairs and cuticle remains unpigmented; no pigmentation of tarsal claws. |
| 11 | Black pigmentation of tarsal claws. |
| 12 | Coarse white hairs are seen for the first time. |
| 14 | No pigmentation of wings; coarse white hairs still present on face and genitalia. |
| 15 | Pigment appears in "eye spots" of forewings; animal not "soft." |
| 16 | Full pigmentation of wings; animal begins to soften due to breakdown of pupal endocuticle. |
| 18 | Animal soft; wings fully pigmented; molting fluid still present. |
| 19 | Resorption of molting fluid begins. |
| 20 | Molting fluid resorbed and replaced by air; elongation and distension of body; moth emerges and expands wings. |

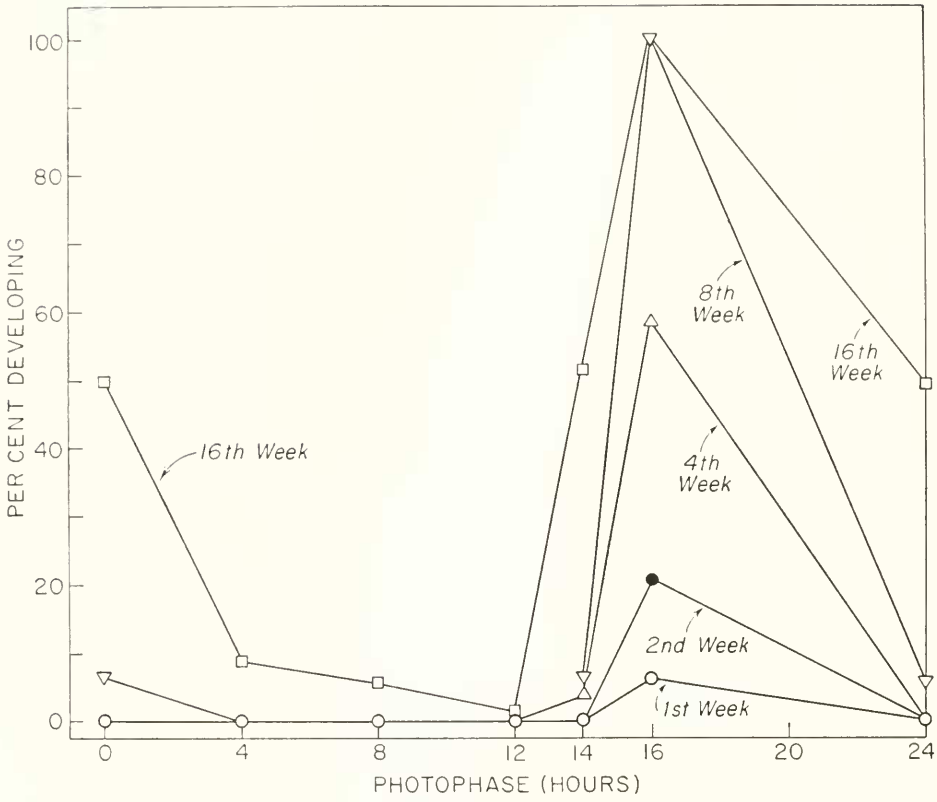


FIGURE 2. The effects of photoperiod on the termination of diapause by unchilled pupae of *A. pernyi* at 25° C. The termination of diapause was recognized or computed in terms of the zero day of adult development.

by the initiation of epidermal retraction, may be recognized with a precision of 4 to 6 hours.

A similar calibration of the diapausing second-brood pupae revealed a slightly faster pace of adult development in that the moths emerged $17 \pm 2$ days after the visible initiation of development.

## 2. *The influence of photoperiod on the termination of diapause*

### A. *Unchilled pupae*

Groups of 100 cocoons and 48 naked pupae were placed at seven different photoperiods at 25° C. The results, summarized in Figure 2, reveal that the 16-hour photophase was most effective in promoting the termination of diapause; after only 4 weeks, over 50% of these individuals showed the initiation of adult development. By contrast, diapause was persistent in the presence of short-day regimens. A 12-hour photophase was especially effective and sustained the diapause of 98% of pupae during the test period of 16 weeks. Attention is also di-

rected to the similar effects of prolonged exposure to continuous light or darkness. Finally, we can state the surprising finding that the response to photoperiod was the same for naked pupae and for those which remained in cocoons.

B. *Chilled pupae*

The preceding experiment was repeated in greater detail, using previously chilled pupae. These individuals were stored at 2–3° C. from the first week of October; for this series of experiments they were removed from storage between November 30 and January 7 and used immediately. Groups of 50 cocoons were placed at ten different photoperiods at 25° C.

The results, recorded in Figure 3, were essentially the same as observed for unchilled pupae, the only major difference being an accelerated response to the regimens which terminated diapause. With these additional data, we now see that the most effective stimulus for the termination of diapause is provided by a 17-hour photophase. And, here again, the 12-hour photophase was most effective in preventing the termination of diapause.

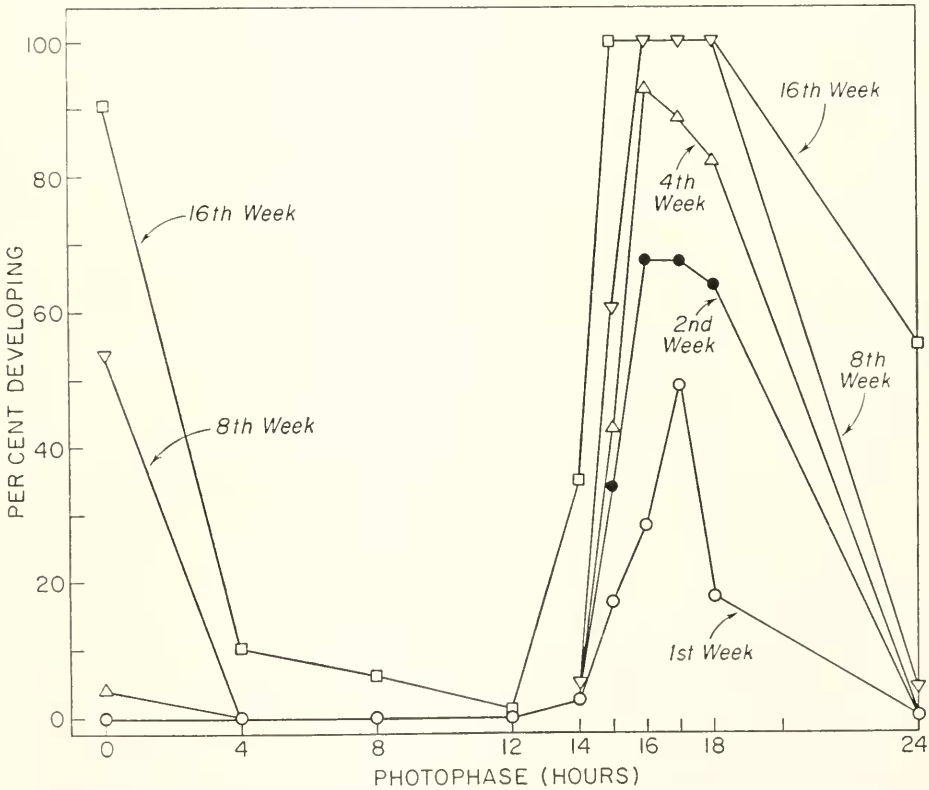


FIGURE 3. The effects of photoperiod on the termination of diapause by previously chilled pupae of *A. pernyi* at 25° C. Diapause is most persistent in the presence of a daily photophase of 12 hours; it is most promptly terminated by a photophase of 17 hours.

3. *The "fine structure" of the photoperiod response*

As is amply demonstrated in Figures 2 and 3, an abrupt transition between "short-" and "long-day" conditions occurs at or near a photophase of 14 hours. This finding was examined in further detail by exposing two groups of 50 unchilled pupae to daily photophases of 13.50 and 13.75 hours, respectively. At the end of eight weeks, 2% of the former group and 22% of the latter group had initiated adult development. This difference signals a discrimination between photophases differing by only 15 minutes.

4. *Effects of preliminary exposure to an 8-hour photophase*

In section 2B of the Results, we observed that the response to photoperiod was accelerated when pupae were first aged at low temperature. A series of experi-

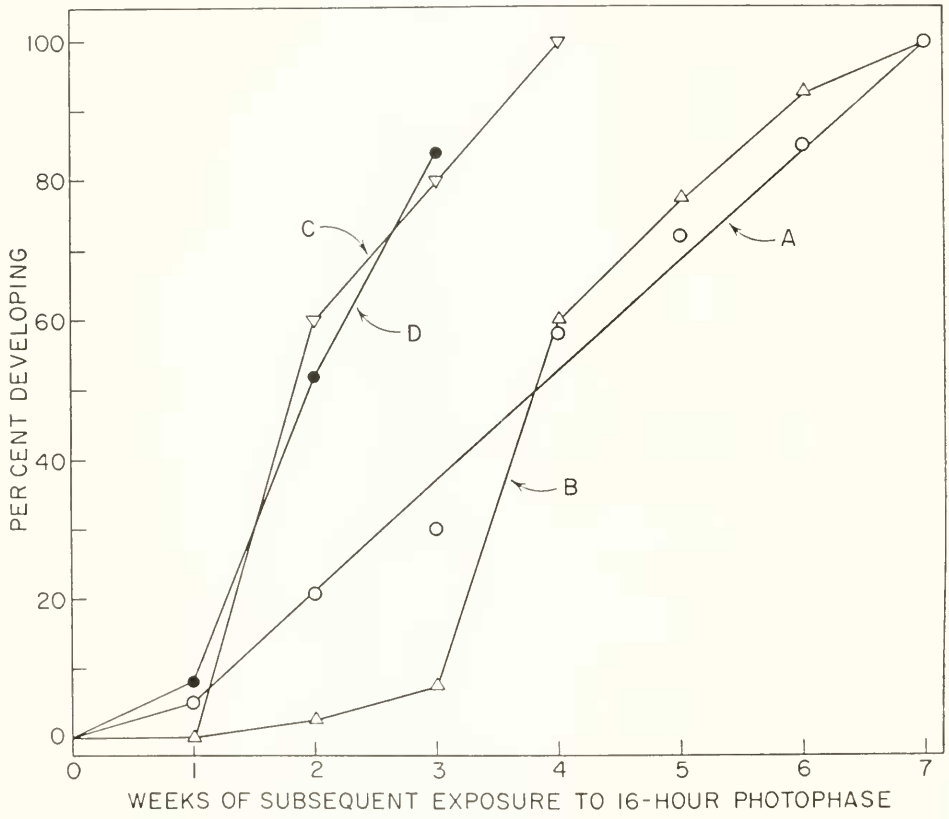


FIGURE 4. Curve A describes the termination of diapause as a function of time; 40 cocoons were incubated at 25° C. in the presence of a long day of 16 hours. In parallel experiments, recorded as Curves B, C, and D, groups of 40 cocoons were first given preliminary exposure at 25° C. to a short-day regimen for 4, 17, and 22 weeks, respectively. The curves describe the termination of diapause after return to the long-day regimen. As noted in Curve B, the preliminary 4-week exposure to the inhibitory photoperiod slowed down the subsequent response. This effect was replaced by a stimulation when the preliminary aging was prolonged to 17 weeks (Curve C) or 22 weeks (Curve D).

ments was designed to test whether the same accelerated response could be induced by aging the pupae at 25° C. in the presence of an inhibitory photophase of 8 hours. To this end, groups of 40 cocoons were first exposed to the 8-hour photophase for 0, 4, 17, and 22 weeks, respectively, and then placed at a 16-hour photophase to induce development.

As summarized (Curve B of Figure 4), the preliminary 4-week exposure to the inhibitory photoperiod slowed down the subsequent response. But when the preliminary exposure was extended to 17 (Curve C) and 22 weeks (Curve D), the inhibition was overcome. When compared to the controls (Curve A), these pupae now showed an accelerated response to the 16-hour photophase reminiscent of that seen after preliminary aging at low temperatures.

5. *Photoperiod gradients*

As described in the section on Methods, 80 unchilled pupae were exposed at 25° C. to an inhibitory 8-hour photophase at one end and a stimulatory photophase of 16 hours at the opposite end. When the experiment was terminated after seven weeks, the results were as follows (see Table II):

TABLE II

*Effect of photoperiod gradients on diapausing pupae at 25°C.*

| Daily photophase (hrs.) | | Number of animals | Cumulative % developing after (weeks) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Head | Abdomen | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 16 | 8 | 40 | 0 | 2.5 | 7.5 | 60.0 | 77.5 | 92.5 | 100 |
| 8 | 16 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

All of the animals initiated development when the head-end was exposed to a daily photophase of 16 hours. None initiated development when the head-end was exposed to the inhibitory photophase of 8 hours.

This shows that the reception of the long-day stimulus occurs at the head-end of the pupa; it also shows that exposure of the abdomen to short-day conditions is ineffective in canceling-out a long-day exposure at the head-end.

6. *Transplantation of photosensitivity*

By previously described techniques (Williams, 1946, 1959), the brains were removed from 26 chilled pupae. Each brain was then reimplanted under a plastic window at the tip of the abdomen. The pupae, now with brains in their hind-ends, were positioned in the early version of the "gradient board" and the latter was placed in a 25° C. incubator programmed for an 8-hour photophase. In this manner each individual was exposed at one end to an inhibitory 8-hour photophase while the opposite end was maintained in continuous darkness. During the two-month terms of the experiment, the results were as follows:

Of the 14 individuals whose brainless anterior ends were exposed to the inhibitory 8-hour photophase, 71% initiated adult development. Of the 12 indi-

viduals whose brain-containing abdomens were exposed to the inhibitory photophase, none initiated development.

So, by the transplantation of the brain to the tip of the abdomen, the sensitivity to photoperiod was likewise transplanted.

*7. The role of the brain in the photoperiodic response*

A. *Brain removal prior to the initiation of adult development*

Brains were removed from 28 diapausing pupae and the brainless individuals were then stored at 25° C. in the presence of a 17-hour photophase. Despite exposure to this most favorable photoperiod, none of the brainless pupae underwent any development (Table III). Most individuals survived for at least six months and finally died without any trace of adult development.

TABLE III

*Effects of brain removal before and after the visible initiation of adult development*

| Day of adult development | Number of animals | Number of moths formed | % forming moths |
|---|---|---|---|
| Prior to zero | 28 | 0 | 0 |
| 0 | 15 | 3 | 20 |
| 1 | 23 | 6 | 26 |
| 2 | 20 | 17 | 85 |

This experiment demonstrates that the brain is indispensable for the initiation of adult development, and that even the most favorable photoperiod becomes completely ineffective in the absence of the brain.

B. *Brain removal after the initiation of development*

A similar group of 58 diapausing pupae was removed from cocoons and exposed to a 17-hour photophase in order to provoke the initiation of adult development. By twice-daily examinations, the zero day of adult development was identified for each individual. Brains were removed on either the zero, first, or second day of adult development and all pupae were then returned to the 17-hour photophase at 25° C. The results are summarized in Table III.

Brain removal on the zero day of adult development completely arrested the further development of 80% of individuals. The same operation performed 24 hours later blocked the further development of 74%. An additional delay of 24 hours (until the "second day of adult development") blocked the development of only 15%.

This experiment shows that in most individuals the brain completes its endocrine function about 60 hours after the visible initiation of adult development.

C. *Effects of photoperiod after the initiation of adult development*

Diapausing second-brood material was used in this experiment. One hundred previously chilled pupae were removed from cocoons, placed at 25° C., and exposed to a 16-hour photophase. On the zero day of adult development, half of the

group was returned to the stimulatory 16-hour photophase; the other half was transferred to an inhibitory 8-hour photophase. Both groups emerged as adult moths after an average of 17 days.

Manifestly, photoperiod loses all its influence on the pace of adult development after the latter has actually begun. The formation of the moth then proceeds at a rate dictated by environmental temperature and without any further reference to photoperiod.

## Discussion

### 1. *The induction and termination of diapause in A. pernyi*

As mentioned in the introduction, the detailed studies of Tanaka (1950a, 1950b, 1950c; 1951a, 1951b) have already demonstrated that the induction of diapause in the Pernyi silkworm is controlled by photoperiod. Tanaka's data on the induction of diapause by photophases within the physiological range of 8 to 18 hours are summarized as the hatched line in Figure 5. For comparison, we record as the unbroken line our data for the photoperiodic control of the termination of pupal diapause in previously chilled Pernyi. It is clear that those photoperiods which are effective in inducing diapause are also effective in stabilizing diapause once the latter has begun. Moreover, the photoperiods which are effective in preventing the onset of diapause are precisely the same as those which provoke the termination of diapause.

The fit of the two sets of data is remarkable if one considers that the investigations were performed independently fifteen years apart. In this connection we may note the strategic position of the photophase of 14 hours as the transition between "short-day" and "long-day" conditions.

The obvious inference is that the same photoperiodic mechanism which controls the induction of diapause is retained by the pupa to control the termination of diapause.

### 2. *The role of the brain in the induction of diapause*

In the Cecropia silkworm the brain is known to play a key role in the induction of pupal diapause. The dormant condition is, in fact, a syndrome of endocrine deficiency due to a failure of the brain to secrete a hormone prerequisite for the initiation of adult development (Williams, 1946, 1952, 1956). This failure is attributable, in turn, to an inactivation of the brain during the prepupal period (Williams, 1952). By contrast, in non-diapausing pupae of *Actias selene*, *Actias luna*, and *Antheraea pernyi*, the pupal brain is not turned off but retains its full activity. Consequently, within a few days after pupation, sufficient brain hormone is secreted to cause the initiation of adult development. If the brain is excised before this activation is complete, then the potentially non-diapausing pupae are forced to diapause (Williams, 1952; Shappirio and Williams, 1957).

Manifestly, the decision to diapause or not to diapause is dictated by what happens to the brain's endocrine activity during pupation. And in the case of *A. pernyi*, as we have seen, what happens to the brain's activity is conditioned by the photoperiods experienced during larval life.

It is important to note that a photoperiod which induces diapause does not immediately shut-off the larval brain. If it did so, pupation would be blocked and

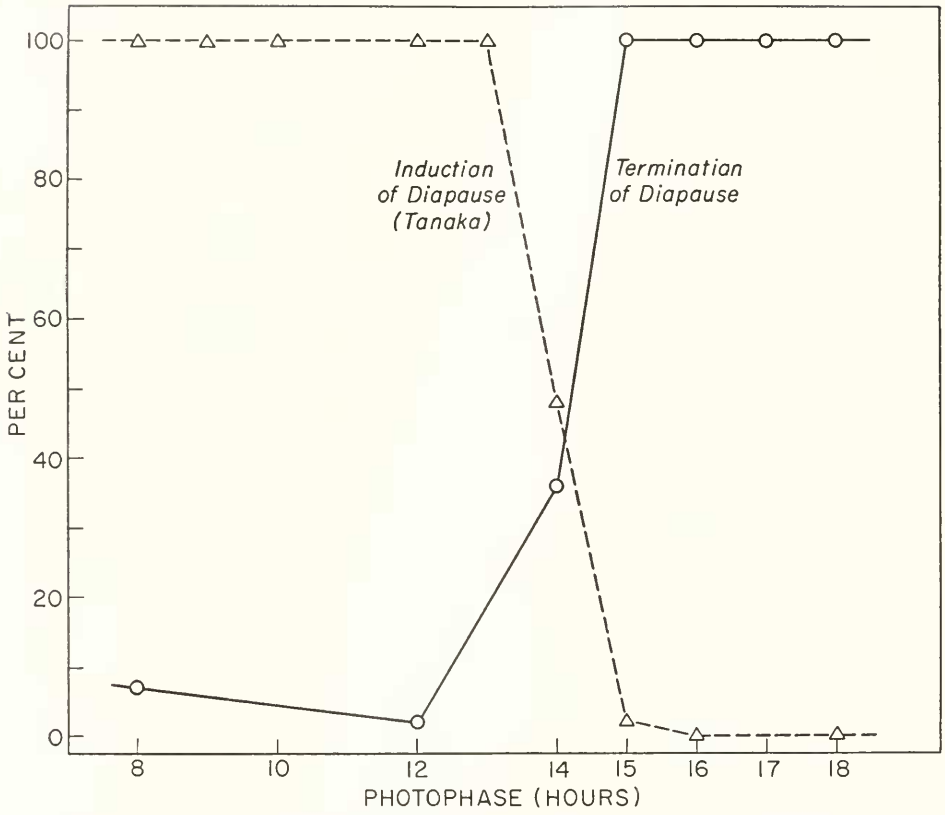


FIGURE 5. The solid line records the effects of photoperiod on the termination of pupal diapause by previously chilled *A. pernyi*. The hatched line shows the influence of photoperiod on the induction of diapause (data of Tanaka). The same short-day conditions which induce pupal diapause are also effective in stabilizing diapause. Moreover, the long-day conditions which prevent pupal diapause are the same as those which cause it to terminate.

one would observe a larval rather than a pupal diapause. The action of photoperiod is to program either the shut-down or the sustained activity of the brain after the latter has secreted sufficient hormone to cause pupation. This state-of-affairs points to some unknown mechanism for the integration and latent storage of daily photoperiod signals accumulated during larval life.

### 3. *The role of the brain in the termination of diapause by photoperiod*

The results of the present study show that photoperiod is ineffective when it acts on brainless pupae. Even a 17-hour photophase is then incapable of causing the termination of diapause. So, by excising the brain, one effectively removes the vehicle for the photoperiod response.

This conclusion is further affirmed by the experiments in which the brain was removed after the initiation of adult development. During the first 60 hours of adult development, the brain hormone completes its tropic action on the pro-

thoracic glands and the brain, itself, is no longer necessary for the continuation of adult development. By this time, photoperiod has become inconsequential and incapable of influencing the further course of events.

We are therefore persuaded that the photoperiod presides over both the onset and the termination of pupal diapause by controlling the endocrine function of the brain.

4. *Direct or indirect action of photoperiod on the brain?*

In a brief report on the photoperiod response of *A. pernyi,* Shakhbazov (1961) called attention to the transparent facial cuticle which overlies the pupal brain. When this zone was coated with black paint, the pupae behaved as if they were in continuous darkness. Shakhbazov concluded that light is transmitted through both the cocoon and the facial "window" to act on some organ in the pupal head.

In the present study this conclusion has been further documented by exposing individual pupae to photoperiod gradients. Long-day conditions promoted the termination of diapause only when they acted on the head-end. In like manner, short-day conditions were effective in maintaining diapause only when they acted on the head-end. By contrast, exposure of the abdomen to either long- or short-day conditions was without any detectable effects.

The cephalic action of photoperiod has been previously described by Lees (1960) in studies of the aphid, *Megoura.* These remarkable experiments have now been extended and reported in full detail (1964). By pin-pointing tiny beams of light through hollow needles or plastic filaments, Lees was able to show that the photosensitivity of the aphid is confined to the head and that the central region of the head is particularly important as a light pathway.

The present study is in full agreement with Lees' findings and provides the first direct evidence that photoperiod acts on the brain itself. Thus, when the brain was excised from the head and reimplanted into the tip of the abdomen, the entire mechanism of the photoperiod response was thereby transplanted to the hind-end. So, in the case of *A. pernyi* the evidence is little short of conclusive that photoperiod acts directly on the brain.

This conclusion is contrary to that which Beck and Alexander (1964a, 1964b) have recently proposed on the basis of their studies of the termination of larval diapause in the European corn-borer, *Ostrinia nubilalis.* In this species, photoperiod is reported to act on certain cells in the mucosa of the anterior region of the hindgut. These cells are said to secrete a brain-stimulating hormone ("proctodone") which under long-day conditions is in phase with a circadian rhythm of brain reactivity; under short-day conditions, proctodone is ineffective because it is secreted out-of-phase with the endogenous brain rhythm. The new hormone is alleged to play a key role in embryonic and postembryonic development, as well as "in non-diapause growth, polymorphism, periodism, and the several forms of diapause" (Beck and Alexander, 1964b).

It is not our present purpose to discuss the new theory in detail. Proctodone has not entered into our calculations for in *A. pernyi,* as we have seen, it is the head-end which is sensitive to photoperiod and, within the head-end, the brain itself. In *A. pernyi,* we have found no trace of the mechanism described by Beck and Alexander.

5. *The endocrine mechanism*

The present study provides the first experimental proof that the photoperiod acts directly on the brain, itself, to control and modulate the secretion of brain hormone. As mentioned above, this conclusion has long been implicit in the pioneering studies of Lees (1955, 1960, 1964) on the photoperiodic responses of aphids.

We shall postpone to a later occasion detailed consideration of how photoperiod acts on the brain to control the secretion of brain hormone. For present purposes, suffice it to say that the minimal brain mechanism presumably includes the following: (1) a pigment for the absorption of appropriate wave-lengths of light; (2) a timing mechanism which counts the hours of darkness (and perhaps also the hours of light); (3) an output from the clockwork-computer to the neurosecretory cells of the brain; and (4) some sort of physiological "needle-valve" for regulating the secretion and translocation of brain hormone.

## Summary

1. In the oak silkworm, *Antheraea pernyi*, photoperiod controls not only the onset of pupal diapause (as previously demonstrated by Tanaka) but also the termination of pupal diapause.

2. At 25° C., short-day conditions (4- to 12-hour photophases) strongly inhibit the termination of pupal diapause; maximal inhibition is by a 12-hour photophase.

3. Long-day conditions (15- to 18-hour photophases) promote the termination of diapause; a 17-hour photophase is the most effective.

4. A 14-hour photophase is transitional between short-days which sustain diapause and long-days which terminate diapause.

5. By various experimental maneuvers, sensitivity to photoperiod was localized in the head-end of the pupa. Short-day illumination of the head-end inhibited the termination of diapause even when the hind-end was exposed to long-day conditions. In like manner, long-day illumination of the head-end was fully effective even when the abdomen received short-day illumination.

6. When the brain was removed from the head and implanted into the tip of the abdomen, the sensitivity to photoperiod was thereby shifted to the hind-end.

7. Additional experiments indicated that the brain is the vehicle for the reception and implementation of photoperiod signals. Brainless pupae are insensitive to photoperiod, while normal pupae are sensitive to photoperiod during the period when development is dependent on the secretion of brain hormone. When this period terminates about 60 hours after the initiation of adult development, photoperiod has lost all its effectiveness.

8. It is concluded that photoperiod acts directly on the brain, itself, to modulate the secretion of brain hormone and thereby to control the termination of pupal diapause.

## Note Added to Proof

Since this manuscript was submitted, additional studies have demonstrated that diapausing pupae of *Antheraea polyphemus* respond to photoperiod in essentially

the same manner as here described for *A. pernyi*. The same appears to be true for diapausing (unchilled) pupae of *Hyalophora cecropia*.

## LITERATURE CITED


Adkisson, P. L., 1964. Action of the photoperiod in controlling insect diapause. *Amer. Nat.* (in press).

Beck, S. D., and N. Alexander, 1964a. Hormonal activation of the insect brain. *Science,* **143**: 478–479.

Beck, S. D., and N. Alexander, 1964b. Proctodone, an insect developmental hormone. *Biol. Bull.,* **126**: 185–198.

Dutky, S. R., M. S. Schechter and W. R. Sullivan, 1962. A lard-can device for experiments in photoperiodism. *J. Econ. Entomol.,* **55**: 575.

Harvey, W. R., and C. M. Williams, 1958. Physiology of insect diapause. XII. The mechanism of carbon monoxide-sensitivity and -insensitivity during the pupal diapause of the Cecropia silkworm. *Biol. Bull.,* **114**: 36–53.

Lees, A. D., 1955. The Physiology of Diapause in Arthropods. Cambridge University Press.

Lees, A. D., 1960. Some aspects of animal photoperiodism. *Cold Spr. Harb. Symp. Quant. Biol.,* **25**: 261–268.

Lees, A. D., 1964. The location of the photoperiodic receptors in the aphid *Megoura viciae* Buckton. *J. Exp. Biol.,* **41**: 119–133.

Shakhbazov, V. G., 1961. The reaction of the length of daylight and the light receptor of the pupa of the Chinese oak silkworm *Antheraea pernyi G. Dok. Akad. Nauk* SSSR, **140**, No. 1: (AIBS) 944–946.

Shappirio, D. G., and C. M. Williams, 1957. The cytochrome system of the Cecropia silkworm. II. Spectrophotometric studies of oxidative enzyme systems in the wing epithelium. *Proc. Royal Soc. London, Ser. B,* **147**: 233–246.

Tanaka, Y., 1950a. Studies on hibernation with special reference to photoperiodicity and breeding of the Chinese Tussar-silkworm. I. *J. Scric. Sci. Japan,* **19**: 358–371. (*In Japanese*).

Tanaka, Y., 1950b. Studies on hibernation with special reference to photoperiodicity and breeding of the Chinese Tussar-silkworm. II. *J. Scric. Sci. Japan,* **19**: 429–446. (*In Japanese*).

Tanaka, Y., 1950c. Studies on hibernation with special reference to photoperiodicity and breeding of the Chinese Tussar-silkworm. III. *J. Scric. Sci. Japan,* **19**: 580–590. (*In Japanese*).

Tanaka, Y., 1951a. Studies on hibernation with special reference to photoperiodicity and breeding of the Chinese Tussar-silkworm. V. *J. Scric. Sci. Japan,* **20**: 132–138. (*In Japanese*).

Tanaka, Y., 1951b. Studies on hibernation with special reference to photoperiodicity and breeding of the Chinese Tussar-silkworm. VI. *J. Scric. Sci. Japan,* **20**: 191–201. (*In Japanese*).

de Wilde, J., 1962. Photoperiodism in insects and mites. *Ann. Rev. Entomol.,* **7**: 1–26.

Williams, C. M., 1946. Physiology of insect diapause: the role of the brain in the production and termination of pupal dormancy in the giant silkworm *Platysamia Cecropia*. *Biol. Bull.,* **90**: 234–243.

Williams, C. M., 1952. Physiology of insect diapause. IV. The brain and prothoracic glands as an endocrine system in the Cecropia silkworm. *Biol. Bull.,* **103**: 120–138.

Williams, C. M., 1956. Physiology of insect diapause. X. An endocrine mechanism for the influence of temperature on the diapausing pupa of the Cecropia silkworm. *Biol. Bull.,* **110**: 201–218.

Williams, C. M., 1959. The juvenile hormone. I. Endocrine activity of the corpora allata of the adult Cecropia silkworm. *Biol. Bull.,* **116**: 323–338.

Williams, C. M., 1963. Control of pupal diapause by the direct action of light on the insect brain. *Science,* **140**: 386.

Williams, C. M., and P. L. Adkisson, 1964. Photoperiodic control of pupal diapause in the silkworm, *Antheraea pernyi*. *Science,* **144**: 569.